

AWARD NUMBER: W81XWH-14-1-0077

TITLE: A Novel Therapeutic for the Treatment and Prevention of Hearing Loss
from Acoustic Trauma

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REPORT DATE: October 2016

TYPE OF REPORT: Final

PREPARED FOR: U.S. Army Medical Research and Material Command
Fort Detrick, Maryland 21702-5012

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REPORT DOCUMENTATION PAGE				Form Approved OMB No. 0704-0188	
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1. REPORT DATE October 2016		2. REPORT TYPE Final		3. DATES COVERED 05/01/2014-07/31/2016	
4. TITLE AND SUBTITLE A Novel Therapeutic for the Treatment and Prevention of Hearing Loss from Acoustic Trauma				5a. CONTRACT NUMBER : W81XWH-14-1-0077	
				5b. GRANT NUMBER	
				5c. PROGRAM ELEMENT NUMBER	
6. AUTHOR(S) Steven Hefeneider; Sharon McCoy E-Mail: steve@13therapeutics.com; sharon@13therapeutics.com				5d. PROJECT NUMBER	
				5e. TASK NUMBER	
				5f. WORK UNIT NUMBER	
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) 13therapeutics, Inc., 4640 SW Macadam Ave., Suite 200D, Portland, OR 97239				8. PERFORMING ORGANIZATION REPORT NUMBER	
9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES) U.S. Army Medical Research and Materiel Command Fort Detrick, Maryland 21702-5012				10. SPONSOR/MONITOR'S ACRONYM(S)	
				11. SPONSOR/MONITOR'S REPORT NUMBER(S)	
12. DISTRIBUTION / AVAILABILITY STATEMENT Approved for Public Release; Distribution Unlimited					
13. SUPPLEMENTARY NOTES					
14. ABSTRACT During this granting period we have demonstrated that our therapeutic peptide, P13/T1, was efficacious in treating permanent and severe noise-induced hearing loss. Using a steady-state model of noise exposure (117 db for 2 hours), T1/P13 was administered one hour after noise using a variety of doses and routes of administration. Both topical (ear drop) and subcutaneous (SQ) routes of administration of T1/P13 resulted in a reduction in hearing loss seen after noise insult, with the most dramatic improvement seen after SQ administration. P13/T1 also demonstrated an improvement in hearing thresholds when given either one or 24 hours before the noise insult. Mass spectrometry studies demonstrated that P13/T1 reaches the inner ear when given either subcutaneously or topically. Gene expression studies show that P13/T1 impacts numerous genes related to noise-induced hearing loss.					
15. SUBJECT TERMS Noise-induced hearing loss; Toll-like receptor (TLR), Auditory brainstem response (ABR); Intra-cellular signaling pathways; tympanic membrane					
16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT Unclassified	18. NUMBER OF PAGES 22	19a. NAME OF RESPONSIBLE PERSON USAMRMC
a. REPORT Unclassified	b. ABSTRACT Unclassified	c. THIS PAGE Unclassified			19b. TELEPHONE NUMBER (include area code)

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1. INTRODUCTION

Hearing loss caused by noise exposure is a significant medical problem, with work-place associated noises such as machinery a frequent cause of hearing loss in both the civilian and military populations. In the military population, acute noise exposure resulting from explosions, blasts, or gunshots, represents an additional risk for hearing loss. Currently there is no treatment for hearing loss caused by noise exposure, and prevention is focused on reducing noise exposure and utilization of hearing protection devices. Recent investigations have identified at least three signaling pathways as being critical in contributing to cochlear hair cell damage or death by apoptosis following noise exposure. Interconnected with these pathways is the Toll-like receptor (TLR) signaling pathway. Our laboratory has recently identified and characterized a novel peptide inhibitor of TLR signaling termed P13. The global hypothesis of this application is that modulation of TLR signaling by P13 will prevent or limit activation of these interconnected signaling pathways that trigger the damage or apoptotic death of cochlear hair cells. Inhibition of these pathways by P13 will prevent/limit cochlear hair cell damage/death following noise exposure, leading to improved hearing.

2. KEYWORDS

Noise-induced hearing loss
Toll-like receptor (TLR)
MAPK/JNK pathway
Reactive oxygen species
Auditory Brainstem Response (ABR)
Intra-cellular signaling pathways
Apoptosis
Cochlear hair cell death
Tympanic membrane

3. ACCOMPLISHMENTS

➤ What were the major goals of the project?

The following were the specific aims for the project:

Aim #1: Determine the preclinical efficacy of P13, when administered after acoustic trauma as a treatment protocol, to limit hearing impairment.

Aim #2: Establish the kinetics of P13 peptide transfer across the tympanic membrane following topical (ear drop) administration and quantify the amount of P13 that reaches the inner ear after acoustic trauma.

Aim #3: Determine the preclinical efficacy of P13, when administered prior to acoustic trauma as a preventative protocol, to limit hearing impairment

Aim #4: Determine the mechanism of action by which P13 limits hearing impairment in models of acoustic trauma

➤ What was accomplished under these goals?

The following experiments were conducted for Specific Aim #1: Determine the preclinical efficacy of P13, when administered after acoustic trauma as a treatment protocol, to limit hearing impairment.

- A) A Research Assistant with several years of experience in conducting noise exposure and ABR experiments was hired.
- B) Experiments were conducted to ensure that the noise-exposure chamber for steady state exposure and the ABR machine were functioning correctly.
- C) Experiments were conducted to finalize the noise-insult parameters in the murine steady state model. Two noise insult levels, 110 db, which we have used in our previous studies, and 117 db, which has been used in other experimental labs were tested, and hearing thresholds measured at both one day and one week post noise. The 110 db model demonstrated hearing loss at 16 and 32 kHz, but not at 4 or 8 kHz, while the 117 db model demonstrated hearing loss at all four frequencies. Based on these studies, we have elected to proceed with 117 db noise exposure (figures 1 and 2).

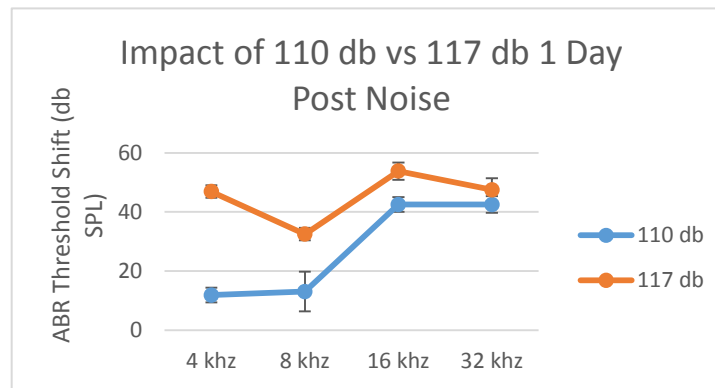


Figure 1. Mice were exposed to either 110 db or 117 db steady-state noise for 2 hours. One hour post noise all animals were administered 10 μ g control peptide topically as ear drops. ABRs were collected at 4, 8, 16 and 32 kHz both before noise insult and one day post noise. The hearing loss data is presented as the post-noise ABR minus the pre-noise ABR at each frequency.

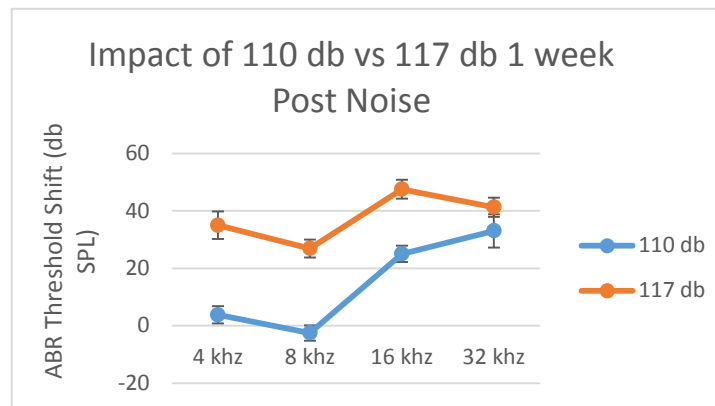


Figure 2. Mice were exposed to either 110 db or 117 db steady-state noise for 2 hours. One hour post noise all animals were administered 10 μ g control peptide topically as ear drops. ABRs were collected at 4, 8, 16 and 32 kHz both before noise insult and one week post noise. The hearing loss data is presented as the post-noise ABR minus the pre-noise ABR at each frequency.

- D) Timing studies were completed in the murine steady state model of noise-induced hearing loss using 117 db noise insult to determine how long the hearing loss due to noise exposure remained in effect. ABRs were completed 1 day, 1 week and 7 weeks post noise insult. For all frequencies tested (4, 8, 16 and 32 kHz), the hearing thresholds were lower (better hearing) at one week as compared to one day post noise exposure, indicating some degree of temporary hearing loss. The hearing thresholds measured at 7 weeks post noise were approximately the same as one week post-noise at all frequencies (permanent hearing loss) (Figure 3).

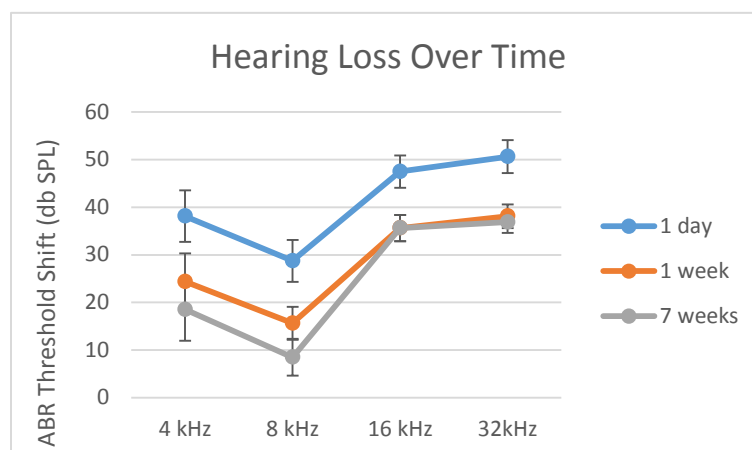


Figure 3. Mice were exposed to 117 db steady-state noise for 2 hours. One hour post noise all animals were administered 10 ug control peptide topically as ear drops. ABRs were collected at 4, 8, 16 and 32 kHz both before noise insult and one day, one week, and seven weeks post noise. The hearing loss data is presented as the post-noise ABR minus the pre-noise ABR at each frequency.

- E) Experiments were conducted to determine which P13 derivative to use for these studies. The anti-inflammatory peptide proposed in this grant, P13, has multiple derivatives. The best derivative, P13/T1 was compared directly to P13 in the noise-induced hearing loss murine steady-state model using 117 db noise insult, and demonstrated greater efficacy at reducing hearing loss due to noise. Based on these studies, we have elected to proceed with P13/T1 for the remainder of the experiments (Figures 4 and 5).

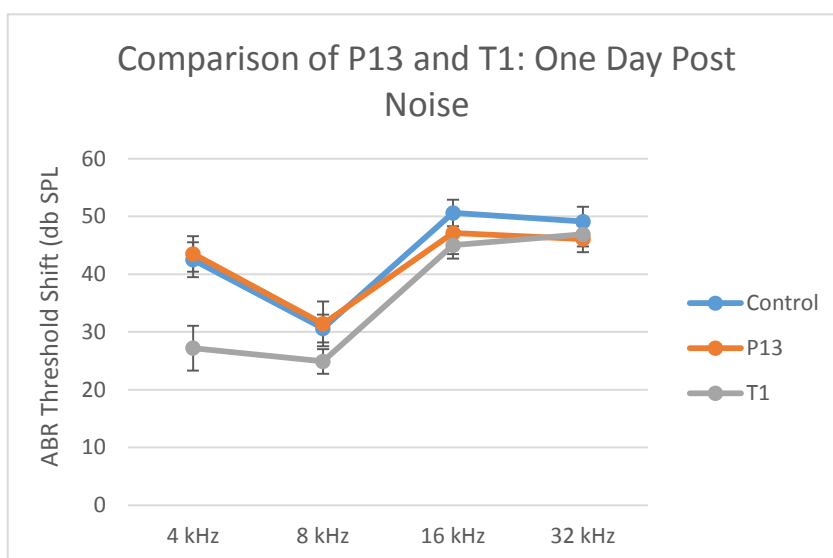


Figure 4. Mice were exposed to 117 db steady-state noise for 2 hours. One hour post noise all animals were administered either 10 μ g control peptide, 10 μ g P13 or 10 μ g P13/T1 topically as ear drops. ABRs were collected at 4, 8, 16 and 32 kHz both before noise insult and one day post noise. The hearing loss data is presented as the post-noise ABR minus the pre-noise ABR at each frequency.

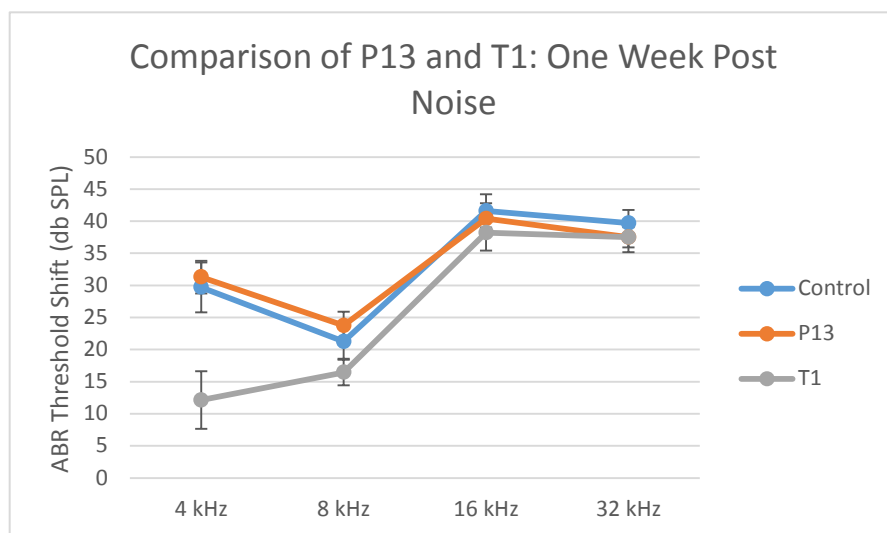


Figure 5. Mice were exposed to 117 db steady-state noise for 2 hours. One hour post noise all animals were administered either 10 μ g control peptide, 10 μ g P13 or 10 μ g P13/T1 topically as ear drops. ABRs were collected at 4, 8, 16 and 32 kHz both before noise insult and one week post noise. The hearing loss data is presented as the post-noise ABR minus the pre-noise ABR at each frequency.

- F) Dosing studies were completed using P13/T1 in the murine steady state model of noise-induced hearing loss using 117 db noise insult, and administering P13/T1 as ear drops. Three doses of P13/T1, 10 μ g, 50 μ g, and 100 μ g, administered topically as ear drops one hour post noise insult were examined. ABRs were completed at one week post noise insult. Both the 10 μ g and 50 μ g doses were effective at reducing the hearing loss seen after noise insult as compared to a control peptide (ANOVA across frequencies for 10 μ g $p=0.0012$, for 50 μ g $p=0.0452$) (Figures 5 and 6). The 100 μ g dose did not reduce hearing loss due to noise.

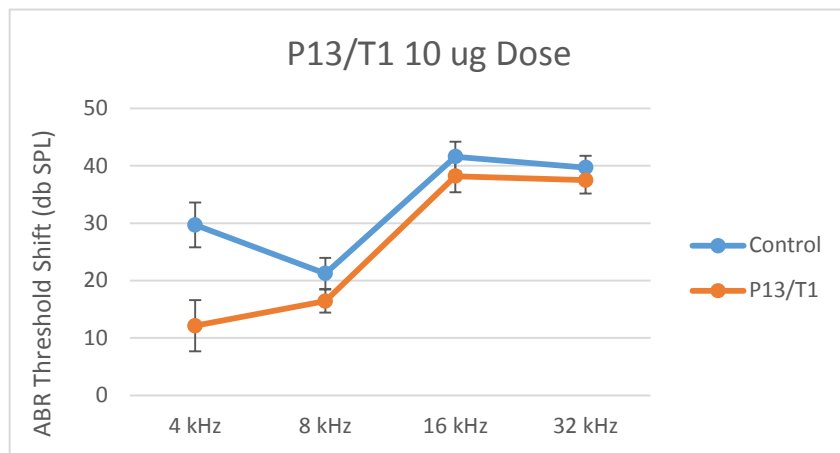


Figure 6. Mice were exposed to 117 db steady-state noise for 2 hours. One hour post noise all animals were administered either 10 μ g control peptide, or 10 μ g P13/T1 topically as ear drops. ABRs were collected at 4, 8, 16 and 32 kHz both before noise insult and one week post noise. The hearing loss data is presented as the post-noise ABR minus the pre-noise ABR at each frequency.

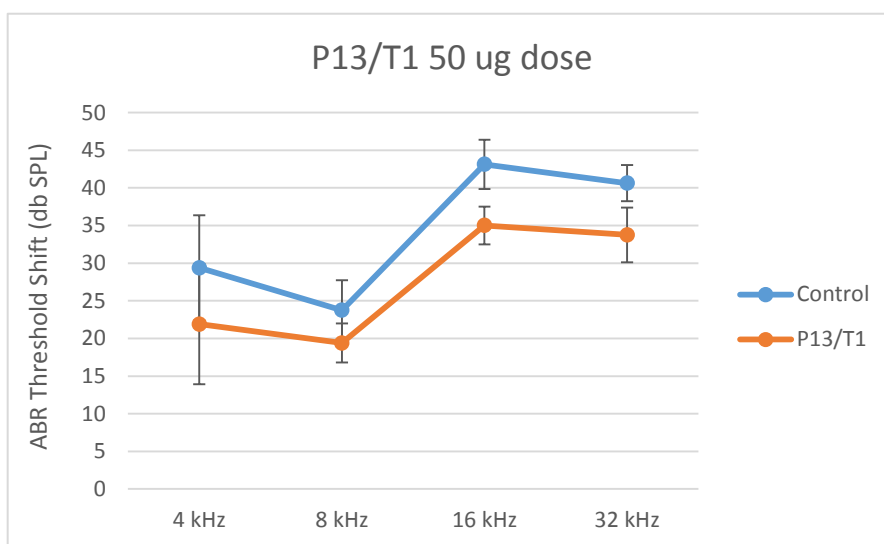


Figure 7. Mice were exposed to 117 db steady-state noise for 2 hours. One hour post noise all animals were administered either 50 μ g control peptide or 50 μ g P13/T1 topically as ear drops. ABRs were collected at 4, 8, 16 and 32 kHz both before noise insult and one week post noise. The hearing loss data is presented as the post-noise ABR minus the pre-noise ABR at each frequency.

- G) Three additional routes of administration for P13/T1, subcutaneous (SQ), trans-tympanic injection, and retro-orbital injection were examined. The purpose of these studies was to both verify the peptide effect and to determine if a different route of peptide administration would be more efficacious than ear drop administration.

- i) For the subcutaneous studies, a dose response was examined. Either 100, 500 or 1000 μg P13/T1 was administered SQ 1 hour post noise insult in the 117 db noise insult steady-state model and compared to control peptide or PBS injected animals. 500 μg P13/T1 demonstrated a reduction in the hearing loss induced by noise (ANOVA across frequencies for 500 μg $p=0.0001$) (Figure 8). The 500 μg dose given SQ demonstrated the best hearing improvement seen to date, for any dose or by any route of administration.

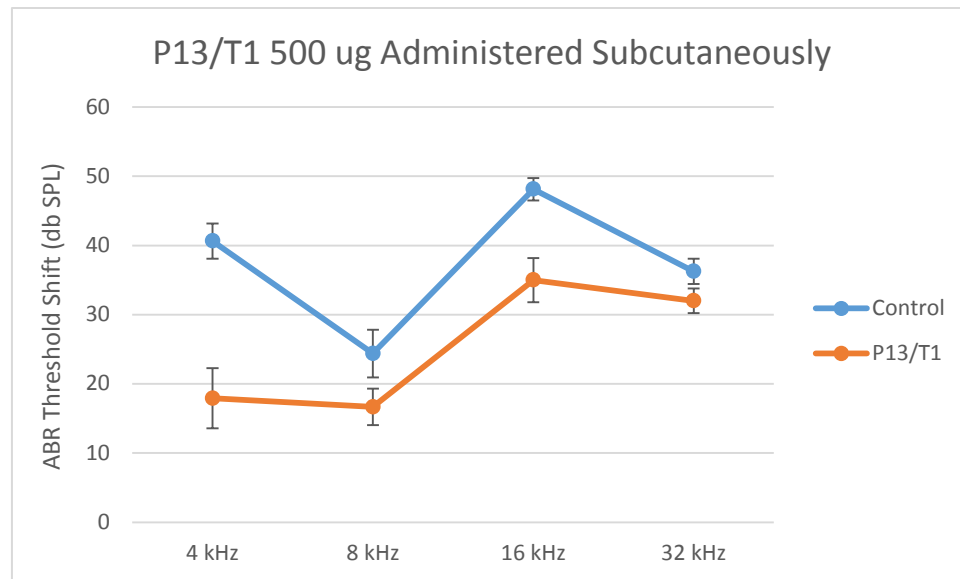


Figure 8. Mice were exposed to 117 db steady-state noise for 2 hours. One hour post noise all animals were administered either 500 μg control peptide or 500 μg P13/T1 administered subcutaneously. ABRs were collected at 4, 8, 16 and 32 kHz both before noise insult and one week post noise. The hearing loss data is presented as the post-noise ABR minus the pre-noise ABR at each frequency.

- ii) P13/T1 was examined using trans-tympanic injections. The injection itself resulted in an inflammatory response, which negated any potential effect on hearing. We speculate this may be due to the anatomy of the mouse ear, as in guinea pigs this administration route has demonstrated efficacy.
- iii) Both P13 and P13/T1 were examined using direct IV injections, administered retro-orbitally. No effect on hearing thresholds was noted using this route of administration.
- H) Multiple administrations of P13/T1 in the steady-state model, both by eardrop and SQ injections were tested.
- i) Two ear drop administrations (10 μg each) given 10 minutes apart, one hour after noise insult were examined. ABRs were completed one week post noise insult. Giving two doses of 10 μg , 10 minutes apart, demonstrated an equal, but not superior, effect on ABR thresholds as compared to one dose.

- ii) Two ear drop administrations (one group 10 µg each dose and one group 50 µg each dose) were given 24 hours apart, with the first dose given one hour after noise insult. No impact on hearing thresholds were seen with this administration protocol.
 - iii) Two SQ doses were given (one group 100 µg each dose and one group 500 µg each dose) were given 24 hours apart, with the first dose given one hour after noise insult. Although there was some impact on hearing thresholds, the effect was not as great as a single SQ dose.
- I) The effect of administering P13/T1 at four separate times after noise insult in the 117 db steady-state model; one hour, six hours, 24 hours and 48 hours after noise was examined. All administrations were 500 µg P13/T1 given subcutaneously (SQ). The mice given peptide at one, six and 24 hours demonstrated improved hearing as compared to control animals, with one hour demonstrating the greatest efficacy (Figures 9,10,11). Mice administered P13/T1 48 hours after noise demonstrated the same amount of hearing loss as controls.

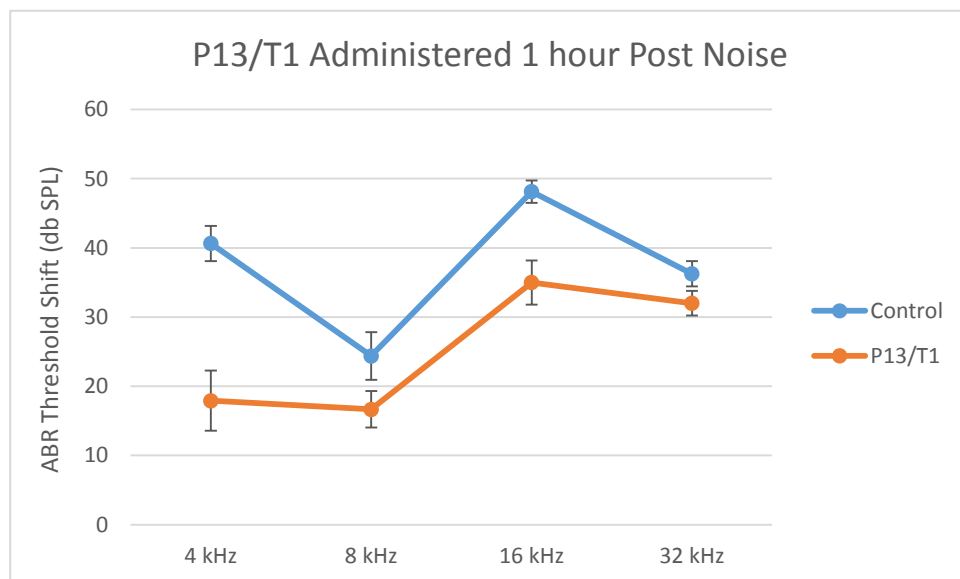


Figure 9. Mice were exposed to 117 db steady-state noise for 2 hours. One hour post noise all animals were administered either 500 µg control or 500 µg P13/T1 subcutaneously. ABRs were collected at 4, 8, 16 and 32 kHz both before noise insult and one week post noise. The hearing loss data is presented as the post-noise ABR minus the pre-noise ABR at each frequency.

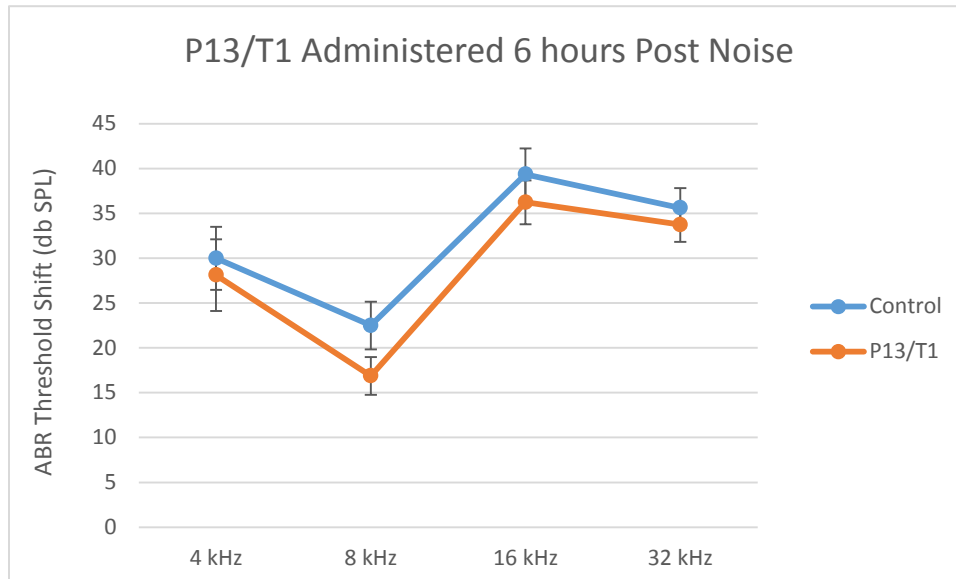


Figure 10. Mice were exposed to 117 db steady-state noise for 2 hours. Six hours post noise all animals were administered either 500 μ g control or 500 μ g P13/T1 subcutaneously. ABRs were collected at 4, 8, 16 and 32 kHz both before noise insult and one week post noise. The hearing loss data is presented as the post-noise ABR minus the pre-noise ABR at each frequency.

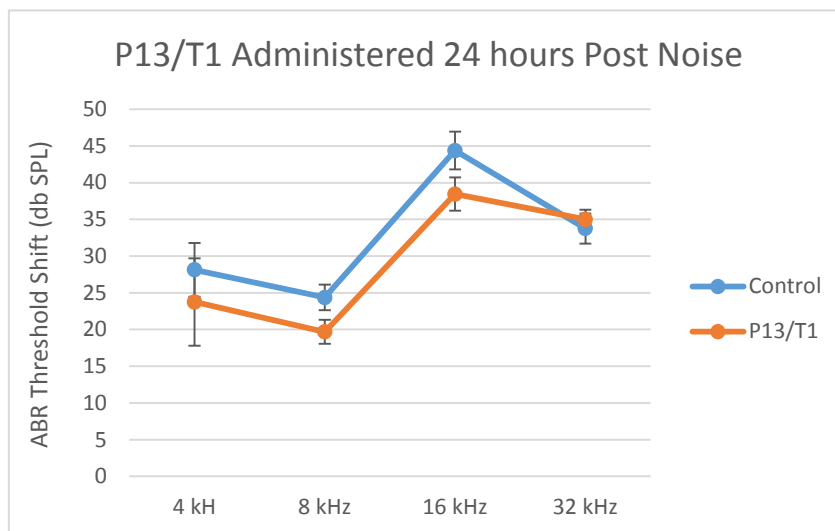


Figure 11. Mice were exposed to 117 db steady-state noise for 2 hours. Twenty-four hours post noise all animals were administered either 500 μ g control or 500 μ g P13/T1 subcutaneously. ABRs were collected at 4, 8, 16 and 32 kHz both before noise insult and one week post noise. The hearing loss data is presented as the post-noise ABR minus the pre-noise ABR at each frequency.

- J) Model development was completed for impulse noise studies. Two noise exposure models are described in this grant, the steady-state model, and the impulse model. To examine the impact of the peptide in this model, the impulse noise chamber was designed, constructed and validated by sound engineers. Model development was then done for the impulse noise model by varying the impulse type (single vs. double), the number of pulses, and the timing of the pulses to which the mice were exposed, and then measuring hearing thresholds at various times post noise exposure. Although numerous exposure conditions were evaluated, this impulse model resulted in a severe, but temporary threshold shift only and hearing levels were quickly restored. The conditions that produced the greatest temporary hearing loss (double impulse, 300 pulses at 1 pair/sec) was chosen for the P13/T1 experiments. Because it was necessary to measure hearing thresholds within 24 hours post noise exposure to document hearing loss, the peptide was tested as a preventative only, and given before impulse noise exposure (see Specific Aim #3).

The following experiments were conducted for Specific Aim #2: Establish the kinetics of P13 peptide transfer across the tympanic membrane following topical (ear drop) administration and quantify the amount of P13 that reaches the inner ear after acoustic trauma:

To complete Specific Aim #2, a method of detecting and quantifying T1/P13 in tissue must first be developed. We developed a method to use mass spectrometry to detect P13 in tissue. Preliminary experiments were completed and we were able to detect T1/P13 in cochlear tissue and demonstrated a dose response. After detection was established, further experiments were done using a stable label internal standard in order to quantify the amount of P13/T1 in cochlear tissue.

After the methods were developed and optimized, the following experiments were completed:

- A) P13/T1 (2 mgs) was injected subcutaneously into mice and mice were sacrificed at 30 min, 1 hr, 2 hrs, 4 hrs, and 7 hrs post injection. Cochlear tissue (2 ears/timepoint) was dissected, and analyzed by mass spectrometry for the amount of P13/T1 present. Results demonstrated that 22 ng P13/T1 were detected per cochlea at 30 minutes, and 8 ng/cochlea detected at 1 hour. By 2 hours the amount detected was 2 ng, and at 4 and 7 hours it was down to 1 or less ng.
- B) P13/T1 (2 µg/ear) was administered by transtympanic injection and mice sacrificed at 15 min, 30 min, 45 min, 1 hr, 1.5 hrs, 2 hrs, and 4 hrs, post injection. Cochlear tissue (2 ears/timepoint) was dissected, and analyzed by mass spectrometry for the amount of P13/T1 present. In this experiment the amount of T1/P13 peaked at 1.5 hours post injection with an average of 291 ng/ear.
- C) P13/T1 (100 µg/ear) was administered topically by ear drop and mice sacrificed at 15 min, 30 min, 1 hr, 2 hrs, 4 hrs, and 7 hrs. The maximum amount (15 ng) reached the inner ear in 15 minutes and the 1, 2, and 4 hr time points also demonstrated approximately 15 ng P13/T1. At 7 hours approximately 3 ng was observed in cochlear tissue.
- D) P13/T1 (10 µg/ear) was administered topically by ear drop and mice sacrificed at 15 min, 30 min, 1 hr, 2 hrs, 4 hrs, and 7 hrs. The amount detected peaked at 1.5 ng/cochlea in 2 hours. At 4 hours the amount was 1 ng, and down to 0.2 ng at 7 hours.

The following experiments were conducted for Specific Aim #3: Determine the preclinical efficacy of P13, when administered prior to acoustic trauma as a preventative protocol, to limit hearing impairment

The work described for Specific Aim #1, sections A, B, C, D, and E are also applicable to this aim.

- A) The impact of P13/T1 on hearing loss was examined at four separate times before noise insult in the 117 db steady-state model. Times examined were one hour, 24 hours, 48 hours and 57 hours before the animals were exposed to noise. All administrations were 500 μ g P13/T1 given subcutaneously (SQ). The 24 hour time points demonstrated improved hearing as compared to control animals (Figure 12). Mice administered P13/T1 one, 48 or 57 hours before noise demonstrated the same amount of hearing loss as controls.

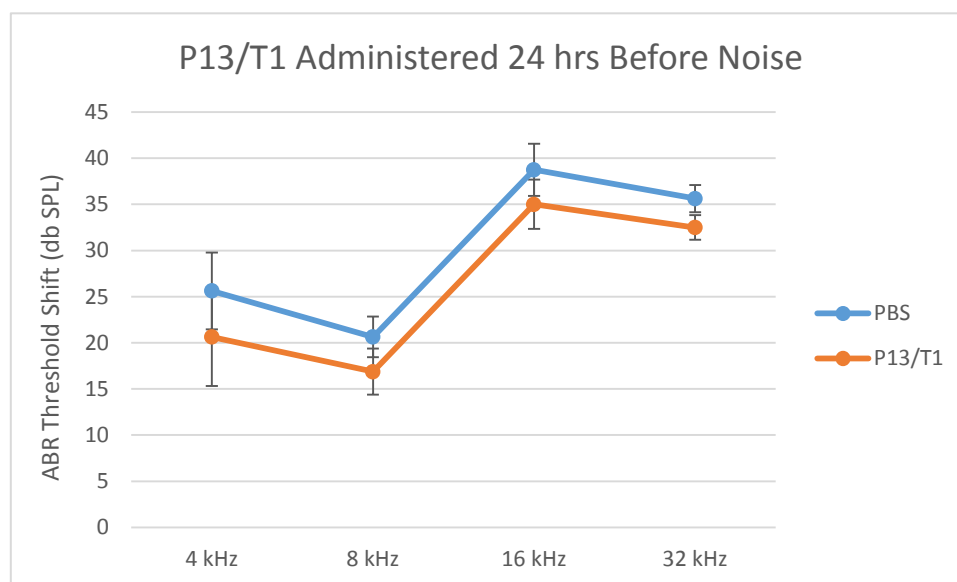


Figure 12. Twenty-four hours before noise exposure all animals were administered either 500 μ g control or 500 μ g P13/T1 subcutaneously. Mice were exposed to 117 db steady-state noise for 2 hours. ABRs were collected at 4, 8, 16 and 32 kHz both before noise insult and one week post noise. The hearing loss data is presented as the post-noise ABR minus the pre-noise ABR at each frequency.

- B) Impulse noise model development was completed (see Specific Aim #1) and either P13/T1 or control peptide was administered subcutaneously (500 μ g) one hour before impulse noise insult, and ABRs read immediately after noise. P13/T1 administration significantly improved hearing at 32 kHz as compared to control peptide. No differences in hearing thresholds were seen between the two at 4, 8 or 16 kHz (figure 13).

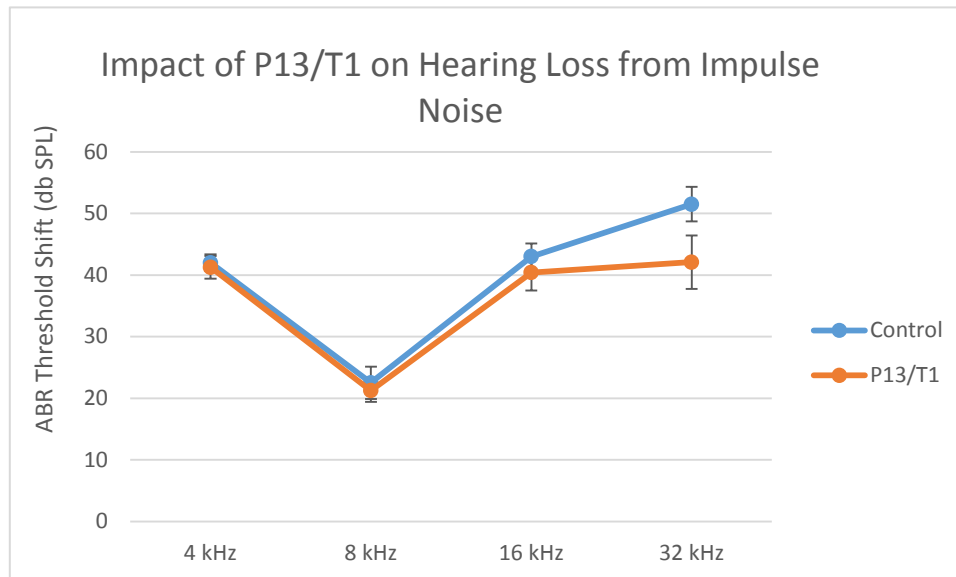


Figure 13. Either P13/T1 or control peptide was administered subcutaneously (500 µg) one hour before impulse noise insult, and ABRs read immediately after noise. The hearing loss data is presented as the post-noise ABR minus the pre-noise ABR at each frequency.

The following experiments were conducted for Specific Aim #4: Determine the mechanism of action by which P13 limits hearing impairment in models of acoustic trauma

Aim #4.1: Determine the gene expression profile resulting from noise exposure and identify genes impacted by P13/T1 treatment following noise exposure.

Before beginning these studies, a part-time Research Assistant with several years of experience with RNA preparation and PCR techniques was hired.

We began PCR studies by first optimizing the inner ear tissue collection process. The RNA preparation, quantification and quality control process were also optimized, as well as the PCR system and array procedures. Data were then collected for four PCR arrays: *i*) oxidative stress and antioxidant defense *ii*) apoptosis *iii*) Toll-like receptor signaling and *iv*) MAP Kinase signaling. Mice were divided into five groups and four of these groups were exposed to noise (117 db for 2 hours). Two of the groups were treated one hour later subcutaneously with 500 µg P13/T1, and two were treated with PBS. One group served as a control and were not exposed to noise. Five hours after noise exposure, inner ear tissue from one P13/T1 group and one PBS group was collected for PCR. Seventeen hours after noise exposure ear tissue was collected from the remaining groups. Numerous genes were impacted by noise at both timepoints. P13/T1 demonstrated a statistically significant effect on several of the genes, and a trend towards reduction of the upregulation due to noise in several others (figures 14 and 15).

Genes impacted by noise exposure (117 db for 2 hours) at 5 hours post exposure:

From the oxidative stress and antioxidant defense array: Fmo2, Hspa1a, Nox1, Ptgs2, Mb, Ng2, Ucp3

From the apoptosis array: Bcl2l10, Casp14, Cd40lg, Cd70, IL10

From the Toll-like receptor signaling array: Cd14, Cebpb, Csf3, Cxcl10, Fos, Hspa1a, Ifng, IL10, IL6, Jun, Lta, Nfkb1a, Ptgs2, TLR7, TLR3, Ticam2, Myd88

From the MAP Kinase signaling array: Cdkn2a, Egr1, Fos, Hspb1, Jun, Sfn

Genes impacted by noise exposure (117db for 2 hours) at 17 hours post exposure:

From the oxidative stress and antioxidant defense array: Hspa1a, Nox1, Ptgs2, Mb, Ngb, Ucp3

From the apoptosis array: Bcl2l10, Casp14, Cd40lg, I10

From the Toll-like receptor signaling array: Cd14, Cebpb, Csf3, Cxcl10, Fos, Hspa1a, Ifng, IL10, Lta, Nfkb1a, Ptgs2, Ticam1, TLR7, TLR3, Ticam2, Myd88

From the MAP Kinase signaling array: Cdkn2a, Egr1, Fos, Hspb1, Jun, Sfn, Mos

T1 Impacts Genes Upregulated 5 hours post Noise Insult

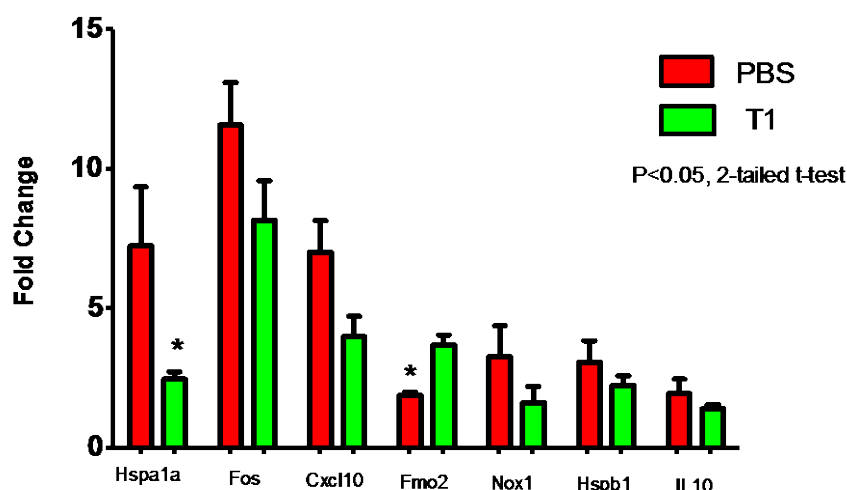


Figure 14. Mice were exposed to noise (117 db for 2 hrs) and treated one hour later subcutaneously with 500 µg P13/T1 or PBS. Five hours after noise exposure, inner ear tissue was isolated and prepared for PCR analysis. Inner ear tissue was analyzed for gene expression using pathway specific PCR arrays (SABioSciences). Noise exposed animals were compared to non-exposed, non-treated controls. The expression level of these controls was set at one, and expression of exposed animals presented as fold-change relative to controls.

T1 Impacts Genes Upregulated 17 hours post Noise Insult

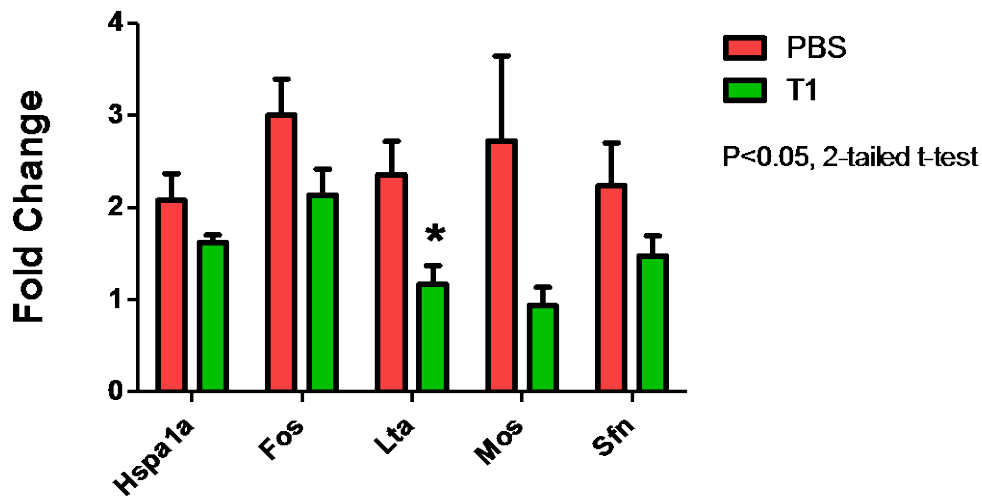


Figure 15. Mice were exposed to noise (117 db for 2 hrs) and treated one hour later subcutaneously with 500 μ g P13/T1 or PBS. Seventeen hours after noise exposure, inner ear tissue was isolated and prepared for PCR analysis. Inner ear tissue was analyzed for gene expression using pathway specific PCR arrays (SABioSciences). Noise exposed animals were compared to non-exposed, non-treated controls. The expression level of these controls was set at one, and expression of exposed animals presented as fold-change relative to controls.

Aim #4.2: Determine the impact of P13/T1 on limiting/preventing cochlear hair cell death following noise exposure

Before beginning the experiments, the collection method and protocol to determine both inner and outer hair cell viability was optimized. Two groups of mice were exposed to steady state noise (117 db) for 2 hours. One hour later one group was injected subcutaneously with 500 μ g P13/T1 and the other group was left untreated. Thirteen days later the animals were sacrificed and the organ of Corti was fixed and dissected free from the bone of the cochlea. After fluorescent immuno-labeling of the cells, they were manually counted in groups. The hair cell groups were correlated with the expected tonotopic frequency of the hair cell location predicted by a standardized mathematical function. The mapping procedure uses software developed by the Eaton Peabody Laboratory at Mass Eye and Ear Hospital. Sixty-one data points were generated for each animal for both inner and outer hair cells counts. Percent viable hair cells was reported for the length of the cochlea from apex to base. All mice, both treated and control, demonstrated no loss of inner hair cell viability. For outer hair cells, the greatest amount of loss was seen at the high frequency end of the cochlea (Figure 16). The mice treated with P13/T1 demonstrated reduced hair cell loss compared to controls, but this difference was not statistically significant (Figure 17).

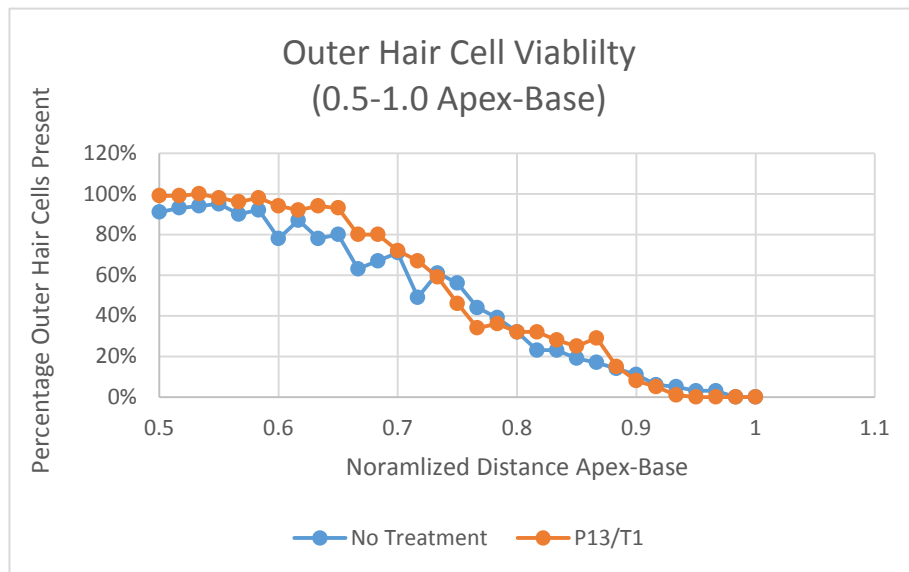


Figure 16. Two groups of mice were exposed to steady state noise (117 db) for 2 hours. One hour later one group was injected subcutaneously with 500 μ g P13/T1 and the other group was left untreated. Thirteen days later the animals were sacrificed and percent viable hair cells determined. The chart shows the high frequency end of the cochlea (0.5 to 1.0 Apex-Base).

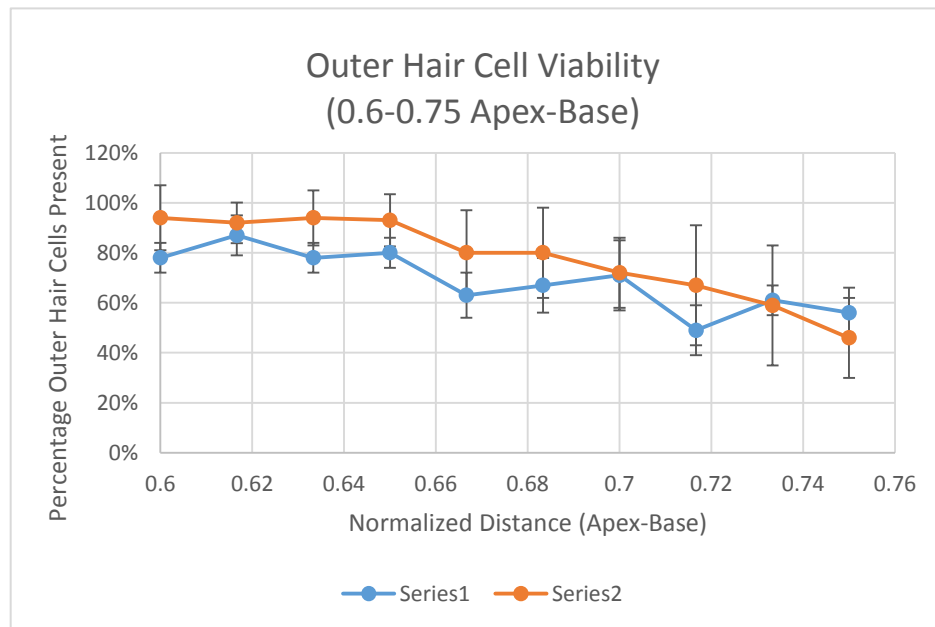


Figure 17. Two groups of mice were exposed to steady state noise (117 db) for 2 hours. One hour later one group was injected subcutaneously with 500 μ g P13/T1 and the other group was left untreated. Thirteen days later the animals were sacrificed and percent viable hair cells determined. The chart shows an isolated part of the high frequency end of the cochlea (0.6 to 0.75 Apex-Base) with error bars.

- **What opportunities for training and professional development has the project provided?**

Nothing to report

- **How were the results disseminated to communities of interest?**

Presentations have been made to interested members of the scientific community at the Department of Otolaryngology at The Oregon Health and Sciences University and Decibel Therapeutics. In addition, the Company has made several presentations to venture groups potentially interested in funding commercial development of this therapeutic for NIHL, including ARCH ventures, Avalon, MPM Capital, and Domain Venture. The Company is in continued discussions with groups potentially interested in funding additional studies and moving the technology forward to commercialization, including the IND studies.

- **What do you plan to do during the next reporting period to accomplish these goals?**

N/A

4. IMPACT

- **What was the impact on the development of the principal discipline(s) of the project?**

The data collected during this granting period adds to the body of knowledge surrounding the mechanism and treatment of noise-induced hearing loss. By demonstrating efficacy in reducing hearing loss due to noise exposure using a TLR inhibitor, we demonstrate that the toll-like receptor signaling pathway plays a role in noise-induced hearing loss, and that a TLR pathway inhibitor may be a valuable therapeutic in treating hearing loss due to noise exposure. The Company has disseminated the results of this study via presentations to a number of Venture Capital groups who potentially may be interested in providing funding for commercial development. The next step in the commercialization process will be to conduct a detailed IND study. The company has explored the costs for this study with three different CRO groups and a major goal of our raising of additional capital is to fund these studies. While we were not successful in obtaining this funding during the current grant period, the Company is actively engaged with Venture groups to move the IND studies forward. IND studies could be initiated as soon as adequate funding is obtained.

- **What was the impact on other disciplines?**

Nothing to report

- **What was the impact on technology transfer?**

Nothing to report

- **What was the impact on society beyond science and technology?**

Hearing loss is a significant problem and efforts to find therapeutic approaches for treatment can provide hope and encouragement for a significant proportion of the population impacted with NIHL.

5. CHANGES/PROBLEMS:

- **Changes in approach and reasons for change**

Nothing to report

- **Actual or anticipated problems or delays and actions or plans to resolve them**

Nothing to report

- **Changes that had a significant impact on expenditures**

Nothing to report

- **Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents**

Nothing to report

6. PRODUCTS

- **Publications, conference papers, and presentations**

Presentations were made to The Oregon Health and Science University and to the management at Decibel Therapeutics. Title: A New Potential Therapeutic to Treat NIHL. Multiple presentations were made to Venture groups including: ARCH, MPM, Avalon, and Domain.

- **Websites or other Internet sites**

Nothing to report

- **Technologies or techniques**

Nothing to report

- **Inventions, patent applications, and/or licenses**

Nothing to report

- **Other products**

Nothing to report

7. PARTICIPANTS AND OTHER COLLABORATING ORGANIZATIONS

- **What individuals have worked on the project?**

Name: Steven Hefeneider, Ph.D.

Project Role: Principal Investigator
Nearest person month worked: 15.6 months
Contribution to Project: Dr. Hefeneider has overseen all phases of the project, including experimental design and result interpretation.

Name: Sharon McCoy
Project Role: Collaborator
Nearest person month worked: 19.1 months
Contribution to Project: Ms. McCoy has been involved with all phases of the project, including experimental design and data analysis

Name: Beth Kempton
Project Role: Research Associate
Nearest person month worked: 10.5 months
Contribution to Project: Ms. Kempton has performed all the noise induction and ABR studies

Name: Fran Hausman
Project Role: Research Associate
Nearest person month worked: 2 months
Contribution to Project: Ms. Hausman has performed the PCR studies

- **Has there been a change in the active other support of the PD/PI9s) or senior/key personnel since the last reporting period?**

Nothing to report

- **What other organization were involved as partners?**

Organization Name: Oregon Health and Science University

Location of Organization: Portland, Oregon

Partner's contribution to the project: 13therapeutics has subcontracted with Oregon Health and Science University to conduct the noise exposure studies and perform the Auditory Brainstem Response testing.

8. SPECIAL REPORTING REQUIREMENTS

The updated Quad Chart is submitted as an appendix.

9. APPENDICES

Quad Chart

A Novel Therapeutic for the Treatment and Prevention of Hearing Loss from Acoustic Trauma

Report Period 05/01/2014-07/31/2016

W81XW-14-1-0077



PI: Steven H. Hefeneider, Ph.D.

Org: 13therapeutics, Inc.

Award Amount: \$1,046,655.00

Study/Product Aim(s)

- 1) Determine the preclinical efficacy of P13, when administered after acoustic trauma as a treatment protocol, to limit hearing impairment.
- 2) Establish the kinetics of P13 peptide transfer across the tympanic membrane following topical (ear drop) administration and quantify the amount of P13 that reaches the inner ear after acoustic trauma.
- 3) Determine the preclinical efficacy of P13, when administered prior to acoustic trauma as a preventative protocol, to limit hearing impairment
- 4) Determine the mechanism of action by which P13 limits hearing impairment in models of acoustic trauma

Exposure to Different Noise Sources



Steady-state



Impulse

Treatment with P13



Audiometric and Histological Readouts

Timeline and Cost

Activities	CY	14-15	15-16	extention
Determine the efficacy of P13, as a treatment protocol, to limit hearing impairment.				
Establish the kinetics of P13 peptide transfer across the tympanic membrane and quantify the amount of P13 that reaches the inner ear after acoustic trauma.				
Determine the preclinical efficacy of P13 as a preventative protocol, to limit hearing impairment				
Determine the mechanism of action by which P13 limits hearing impairment in models of acoustic trauma				
Estimated Budget		\$472,477	\$574,178	0

Goals/Milestones

CY14-15 Goals – 1) Determine the efficacy of P13, as a treatment protocol, to limit hearing impairment and 2) Determine the preclinical efficacy of P13, when administered prior to acoustic trauma as a preventative protocol, to limit hearing impairment 3) Determine the mechanism of action by which P13 limits hearing impairment in models of acoustic trauma

CY15-16 Goals – 1) Complete the impulse model studies, 2) Complete the kinetic studies 3) Determine the preclinical efficacy of P13/T1 as a preventative protocol, to limit hearing impairment and 4) Determine the mechanism of action by which P13/T1 limits hearing impairment in models of acoustic trauma

All goals/milestones completed

Budget Expenditure to Date

Projected Expenditure: \$1,046,655.00

Actual Expenditure: \$1,046,655.00

Updated: 10/06/2016